

Fig. 1



Fig. 2

Fig. 3

Sequence consensus	-35 ▽ tcTTGACat t	-10 ▽ t tg TAtAaT	Tetracycline resistance (μg/ml)
native (pBR322) TTC	TCATGTTTGACAGCTTTATC	ATCGATTAAAGCTTTTAAATGC	40
A,C,G,T substitution (pRAN4)			
pB68 ...	GTGCAGAAACGCCGACAGGGGAAAGAACTGCCGCTTGAC	AT-----	40
pB63 ...	GGAGCCGCCGATTCG	-----	2
pB65 ...	AAGGCAGGGGGGGCGAC	AT-----	2
pB69 ...	GGCGATGCTTTCCAGTTAGATTAG	-----	6
pB810 ...	CCATGCAATA	-----	2
pB813 ...	TTCCGGTGC	-----	4
C,G,T substitution (pRAN3)			
pB79 -TC	TTGGCGCGCGTCGCTTG	A-----	50
pB721 ...	GCCCCCTTTTCTCCCTTG	A-----	60
pB7R3 ...	CGTCCCTGCCTTGGCTTGT	----- TCC	30
pBA1 ...	GGGTGTCGGTCCCGGTGTCTTC	A-----	6
pBA2 ...	CGTGGCGCCGCTGCTTTTC	-----	6
pBA3 ...	GTTTCGCTTCCGGCGTGC	-----	4
pBA4 ...	CGGTGGCGCGCTGTGTCGG	-----	2
pBA5 ...	GGCGGCTCTCCCGCTCGTT	-----	2
pBA6 ...	GGCGGCTGCGGCGGC	-----	2
pBA7 ...	GCCCTGCTTGGTGTCTTGTCCGCC	-----	2
pBA8 ...	TCCTCGGCTGGCCTTCGGG	-----	10
pBA9 ...	TGTTGCTGCGCGCCCG	CGAGAGT-----	2
pBA10 ...	GTGGCGCGCGGCTGGGCTCGG	-----	4
deletion (pBdEC) ...		-----	4

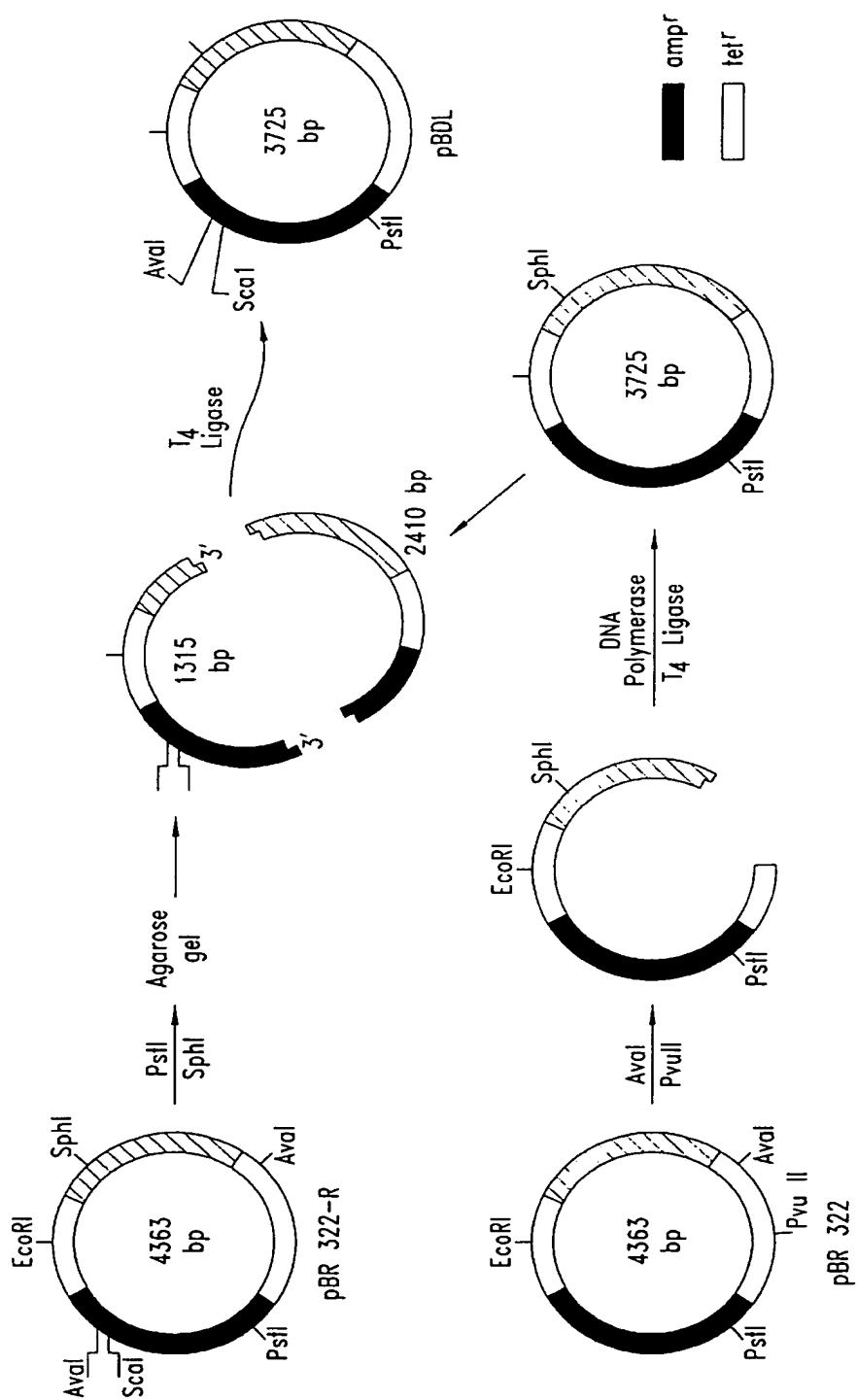


Fig. 4

Step I: Selection of Non-Producer Strain

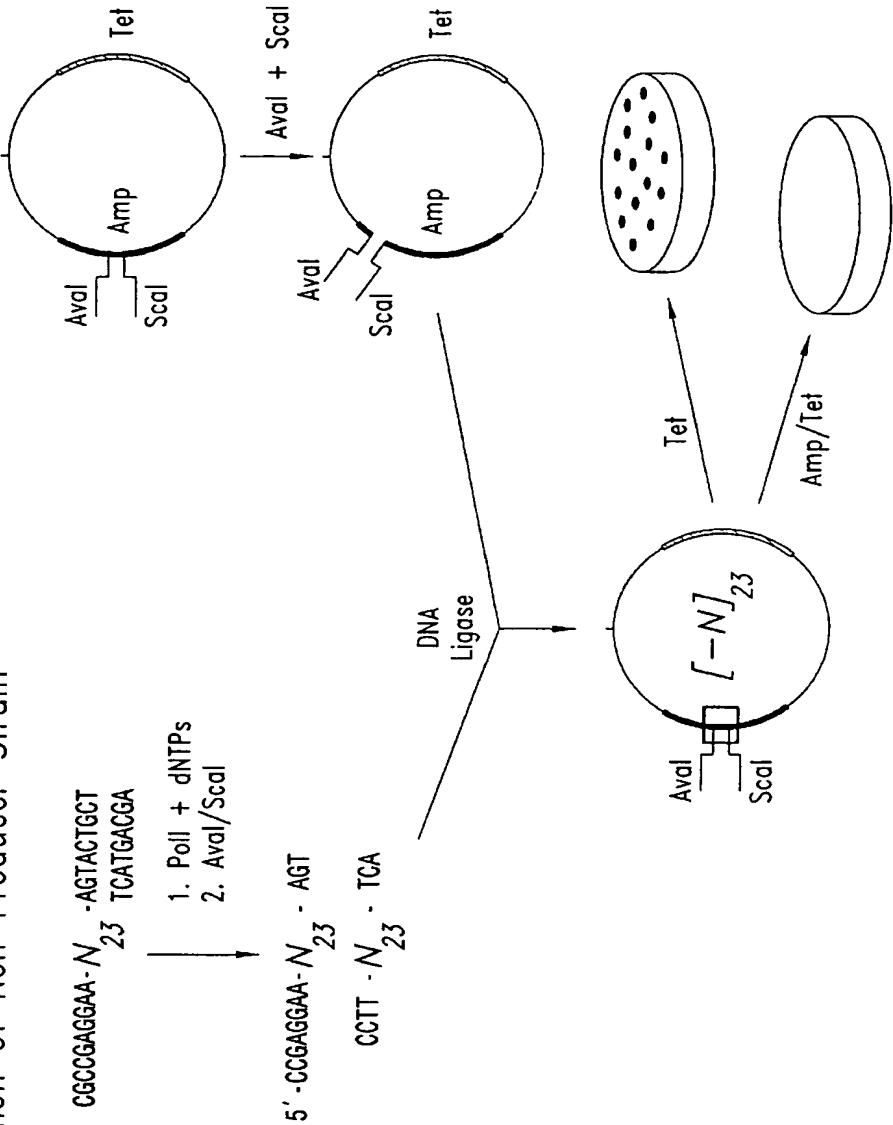


Fig. 5A

Step II: Selection for Carbenicillin Resistance

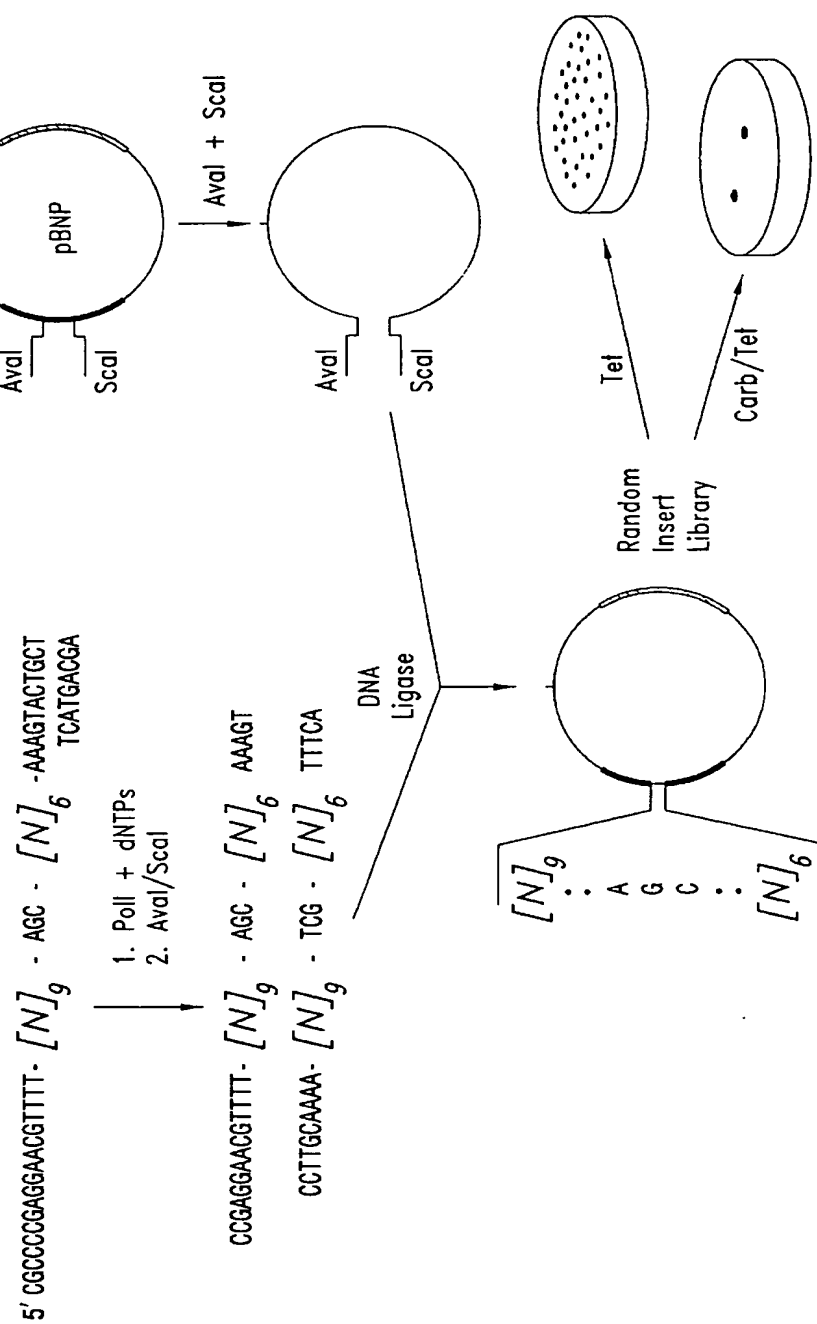


Fig. 5B

Amp (wt)	CGT	TTT	CCA	ATG	ATG	AGC	ACT	TTC	AAA
	Arg	Phe	Pro	Met	Met	Ser	Thr	Phe	Lys
Non Producer	CGT	<u>CAT</u>	<u>TTT</u>	<u>CTG</u>	<u>GGT</u>	<u>GTC</u>	<u>GTT</u>	CAT	CA
	Arg	<u>His</u>	<u>Phe</u>	<u>Leu</u>	<u>Gly</u>	<u>Val</u>	<u>Val</u>	His	
Mutant #1	CGT	TTT	CCC	<u>GTC</u>	ATG	AGC	<u>ATC</u>	<u>ATC</u>	AAA
	Arg	Phe	Pro	<u>Val</u>	Met	Ser	<u>Ile</u>	<u>Ile</u>	Lys
Mutant #2	CGT	TTT	CCG	ATG	<u>CTT</u>	AGC	<u>ACA</u>	<u>ATA</u>	AAA
	Arg	Phe	Pro	Met	<u>Leu</u>	Ser	Thr	<u>Ile</u>	Lys
Mutant #3	CGT	TTT	<u>GCC</u>	<u>CTC</u>	<u>AAT</u>	AGC	<u>ACA</u>	<u>TTT</u>	AAA
	Arg	Phe	<u>Ala</u>	<u>Leu</u>	<u>Asn</u>	Ser	Thr	Phe	Lys
Mutant #4	CGT	TTT	CCT	<u>GTG</u>	<u>TGT</u>	AGC	<u>ACG</u>	<u>CAT</u>	AAA
	Arg	Phe	Pro	<u>Val</u>	<u>Cys</u>	Ser	Thr	<u>His</u>	Lys
Mutant #5	CGT	TTT	CCA	<u>CAA</u>	<u>TTG</u>	AGC	<u>ACC</u>	<u>CAC</u>	AAA
	Arg	Phe	Pro	<u>Gln</u>	<u>Leu</u>	Ser	Thr	<u>His</u>	Lys
Mutant #6	CGT	TTT	CCC	<u>CTT</u>	<u>TCT</u>	AGC	<u>CAC</u>	<u>CGT</u>	AAA
	Arg	Phe	Pro	<u>Leu</u>	<u>Ser</u>	Ser	<u>His</u>	<u>Arg</u>	Lys
Mutant #7	CGT	TTT	CCC	<u>ATA</u>	<u>CTA</u>	AGC	<u>CCA</u>	<u>TCT</u>	AAA
	Arg	Phe	Pro	<u>Ile</u>	<u>Leu</u>	Ser	<u>Pro</u>	<u>Ser</u>	Lys

Fig. 6